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学位授与の題目	Syntheses of Phycocyanobilin Derivatives for Analysis of Chromophore Function in Phytochromes（フィトクロム発色団の機能解明を目指したフィコシアノビリン誘導体の合成）
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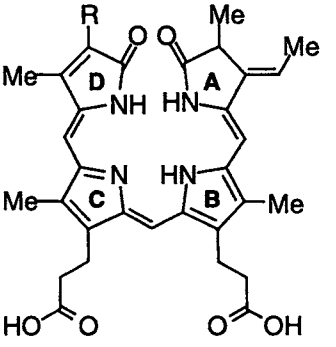
学 位 論 文 要 旨

Abstract Total syntheses of phycocyanobilin (PCB) and its derivative bearing a photoreactive group at D-ring were accomplished by developing a new and efficient method for the construction of A/B-ring component. It consists of the Wittig-type new coupling reaction of 4-(1-methoxyethyl or 1-tosylethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one and a 2-formyl pyrrole derivative in the presence of $^{n}Bu_3P$ and a base, followed by reduction with aluminum amalgam and subsequent acid or base treatment.

Furthermore, to reveal the structural requirement of the chromophore for spectral property of phytochrome B using *in vitro* assembly method, eleven PCB derivatives have been synthesized. Their adducts with *Arabidopsis* phytochrome B apoprotein (PHYB) were investigated spectrophotometrically. In addition, a photoreactive group has been successfully introduced to the D-ring for photoaffinity study which is now in progress.

Biliproteins such as phycocyanin and phytochrome, which contain the bile pigments as chromophoric units, exist in plants. The chromophores named phycocyanobilin (PCB) and phytochromobilin (PΦB) are linear tetrapyrrole derivatives and covalently bonded to their apoproteins at A-ring. Even though such bile pigments, phycobilins, could be isolated from natural sources, the knowledge of the relationship between the structure of synthetic pigments and biochemical properties of the biliproteins obtained by combining them with an apoprotein is quite interesting and important to reveal the precise function of the phycobilins. The recent development in gene technology have made it possible to assemble the chromophores such as PCB and PΦB with the apoprotein obtained by



R = Ethyl, phycocyanobilin (PCB)

R = Vinyl, phytochromobilin (PΦB)

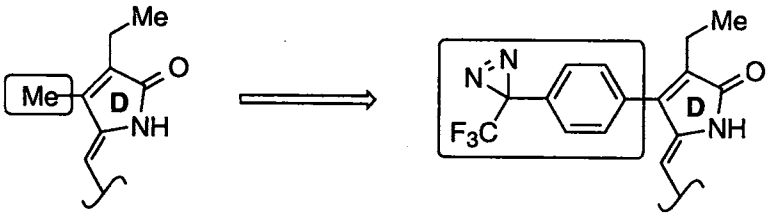
over-expression of the corresponding cDNA in bacteria and yeast. PCB has been often used as a substitute for the natural chromophore PΦB in phytochrome reconstitution experiments. Moreover, the photophysical and photochemical properties of wild type phytochrome are quite similar to those of the reconstituted chromoprotein containing PCB.

Even though there are reports for the syntheses of phycobilin ester derivatives, most of the published works in this area for the synthesis of A/B-ring component have been carried out by utilizing either the Eschenmoser's sulfide contraction, the thio-Wittig coupling, or the photochemical rearrangement of *N*-pyrroloenamide. These methods provide viable routes to the required A/B-ring component, however, the former two usual methods require the removal of a meso-carboxylic ester group at the later stage in the total syntheses of phycobilins. Especially phytochromobilin or its derivatives bearing a vinyl group at D-ring are extremely unstable under acidic conditions, and very difficult to synthesize according to the methods mentioned above.

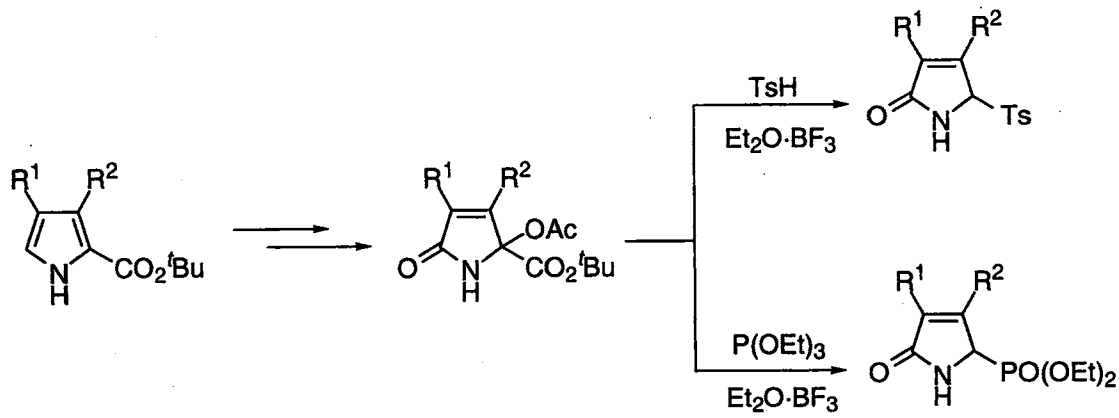
Therefore, it was required to develop a new methodology for the synthesis of A/B-ring component toward the total syntheses of free PCB derivatives applicable for reconstitution experiment.

In the present work, the following achievements were accomplished, namely (1) synthesis of D-ring of phycocyanobilin (PCB) bearing a photoreactive group, (2) an efficient method for the construction of the A/B-ring component toward total syntheses of PCB and its photoreactive derivative, (3) regioselective syntheses of 3,4-disubstituted 5-tosylpyrrolinones and 3,4-disubstituted 1,5-dihydro-5-oxo-2*H*-pyrrol-2-yl-phosphonates from the corresponding 2-pyrrolecarboxylic acid ester derivatives, (4) syntheses of free acid forms of PCB regioisomers to investigate the role of each propanoate side chain, (5) modification of side-chains in D-ring to investigate the spatial and chemical environment around D-ring in phytochrome, (6) introduction of a photoreactive group to D-ring of PCB with a tether for a photoaffinity study, and (7) *in vitro* assembly of phytochrome B apoprotein with chemically synthesized PCB derivatives.

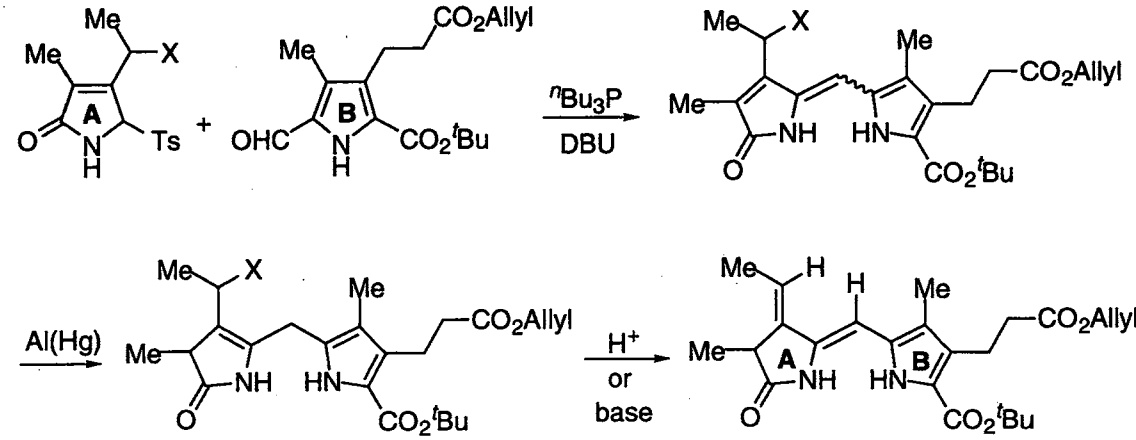
The synthesis of PCB derivative bearing a photoreactive group at D-ring was at first investigated as described in Chapter 2. The carbene generating 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl group was introduced to the D-ring instead of the methyl group in PCB as shown below.



In Chapter 3, a new method for the construction of A/B-ring component utilizing 4-(1-methoxyethyl or 1-tosylethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one was described. The 3,4-disubstituted 5-tosylpyrrolinones and 3,4-disubstituted 1,5-dihydro-5-oxo-2*H*-pyrrol-2-yl-phosphonates useful as a D-ring precursor were both synthesized from the corresponding 2-pyrrolecarboxylic acid esters as shown in the following.

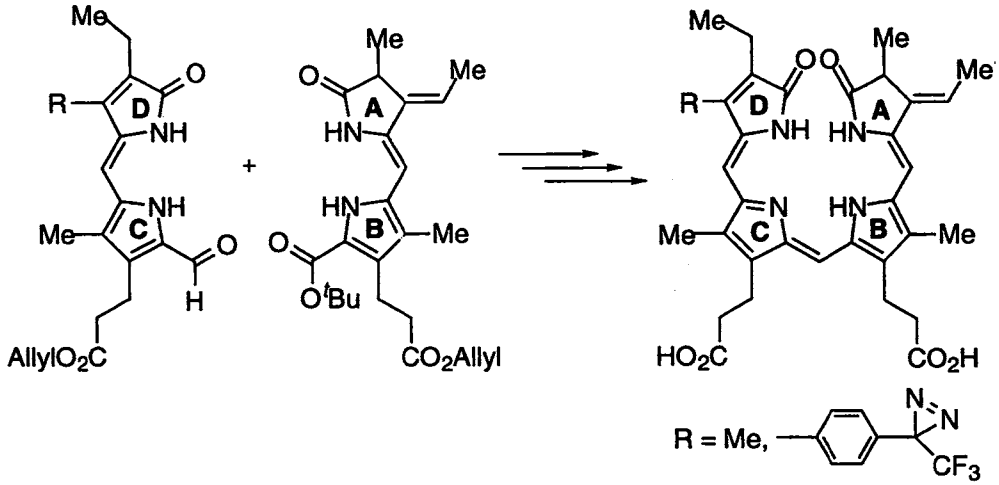


The precursor of A-ring was prepared introducing a leaving group (X) to C-3 ethyl group of the 5-tosylpyrrolinone in good yield. The A-ring derivatives (X = methoxy or tosyl) thus obtained were coupled with a formyl pyrrole as B-ring according to our Wittig-type coupling reaction in the presence of $^{n}Bu_3P$ and a base in CH_2Cl_2 to afford the coupling products as a mixture of (*E*)- and (*Z*)-isomers in good yield. The resulting compounds were reduced with aluminum amalgam, and the intermediates were treated with an acid or a base to give the desired A/B-ring component via elimination of methanol or *p*-toluenesulfinic acid as a single (*Z*)-isomer (confirmed by NOE measurement) in good yield as shown below.



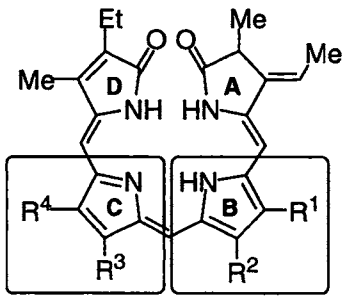
The A/B-ring component was coupled with C/D-rings available by a similar Wittig-type reaction to synthesize free acid forms of PCB and its photoactivatable derivative.

As described above, the Wittig-type coupling reaction proved to be useful not only for the preparation of the C/D-ring component but also for the construction of A/B-ring component by introducing an eliminating group like methoxy or tosyl group into the precursor of A-ring.



In Chapter 4, to investigate the ligation of a chromophore to apophytochrome B (PHYB) and photoreversible spectral change of the holophytochrome B, synthesis of regioisomers of PCB with respect to methyl and propanonic acid substitutes of B- and C-rings was described.

The substituted pyrrole derivatives which are common to B- and C-rings, were prepared starting from nitroolefins and *t*-butoxycarbonylmethyl isocyanide in the presence of DBU, and they were converted to the corresponding ylides as B-ring or α-formyl derivatives as C-ring by Vilsmeier-Haack reaction in high yields. The B- and C-rings were coupled with A-ring and D-ring to afford the corresponding A/B- and C/D-ring components, respectively, and they were coupled toward the syntheses of free acid forms of regioisomers of PCB.



R^1, R^4 = Me, R^2, R^3 = $(CH_2)_2CO_2H$

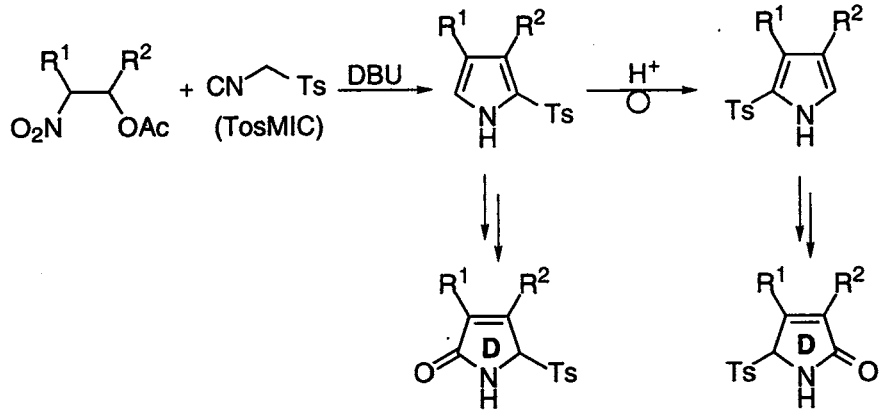
R^1, R^3 = Me, R^2, R^4 = $(CH_2)_2CO_2H$

R^2, R^4 = Me, R^1, R^3 = $(CH_2)_2CO_2H$

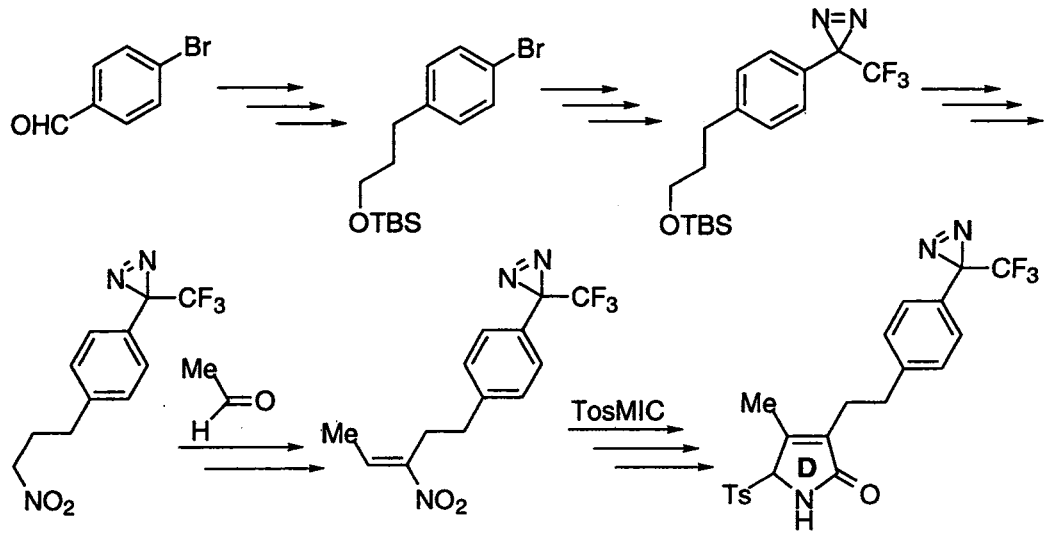
R^2, R^3 = Me, R^1, R^4 = $(CH_2)_2CO_2H$

In Chapter 5, to investigate the spatial and chemical environment around D-ring in phytochrome, syntheses of PCB derivatives bearing a longer alkyl chain like *n*-propyl, *n*-pentyl, *n*-octyl or 2-acetoxyethyl group at C-17 or C-18 position of D-ring were described.

The starting 3,4-disubstituted 2-tosylpyrroles were readily prepared from β-acetoxynitroalkanes and *p*-toluenesulfonylmethyl isocyanide (TosMIC), and the tosyl group was rearranged from 2- to 5-position by treatment with an acid. The pyrrolinone derivatives as D-rings were prepared regioselectively by bromination of the 2-tosylpyrroles and subsequent acidic hydrolysis of the resulting α-bromopyrroles according to the method developed in our laboratory. The pyrrolinone derivatives thus obtained were coupled with a formyl pyrrole to afford the corresponding C/D-ring components, which was further coupled with A/B-ring component toward the syntheses of free acid forms of PCB derivatives.



In Chapter 6, the preparation of 3,4-disubstituted 5-tosylpyrrolinone derivative bearing a photoreactive group was described toward the total synthesis of PCB derivative as a photoprobe. The pyrrolinone derivative was synthesized regioselectively by bromination and subsequent acidic hydrolysis of the corresponding 3,4-disusbtituted 2-tosylpyrrole derivative, which was obtained by cyclization of nitroolefin bearing a photoreactive group and TosMIC in the presence of DBU. The 1-nitro-3-{4-[(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}propane as a starting material was prepared from 4-bromobenzaldehyde. The total synthesis was achieved by employing (1) the coupling of C- and D-rings by our Wittig-type reaction, (2) condensation of A- and B-rings based on the thio-Wittig reaction, (3) coupling of the A/B- and C/D-ring components under acidic conditions, (4) deprotection of allyl ester group using Pd catalyst.



Finally, in Chapter 7 the result of the *in vitro* assembly of phytochrome B apoprotein (PHYB) with the chemically synthesized PCB derivatives was described.

As mentioned above, the present studies on a new method for the synthesis of A/B-ring components, synthesis of D-ring according to an alternative method, and syntheses of chemically modified free acid forms of phycocyanobilin (PCB) derivatives, made it possible to analyze structural requirement of the chromophore for spectral property of phytochrome B. Introduction of photoreactive group to D-ring of PCB has been also achieved for a photoaffinity study.

学位論文審査結果の要旨

提出された当該学位論文に対し，各審査委員が参考論文等の関連資料の検討を含めて審査を行い，さらに平成12年8月7日の口頭発表における質疑応答（最終試験に代える）の結果を踏まえて，同日開催された審査委員会において最終審査を行い，以下の通り判定した。

本論文は，植物の発生や成長，分化等の形態形成を制御している光受容色素蛋白質＝フィトクロムの発色団（フィトクロモビリン）の機能解明を目指して，類似の開環状テトラピロール骨格を有するフィコシアノビリン（PCB）及びその誘導体の新規簡便合成法の開発とアポ蛋白質との再構成実験の結果について述べている。

すなわち，(1)A／B環部分の新規一般合成法の開発，(2)B，C環側鎖のメチル基及びプロピオン酸残基に関する位置異性体の合成，(3)D環17位及び18位への長鎖アルキル基の導入，さらに，(4)光アフィニティ解析を目的としたD環17位及び18位への光活性基の導入，について検討し，いずれも成功に導いている。得られた遊離のPCB誘導体は，組み換え蛋白質として発現させたシロイヌナズナのフィトクロムBアポ蛋白質（PHYB）と試験管内で再構成を行い，発色団の結合および得られたフィトクロムBホロ蛋白質（PhyB）の光可逆的吸収変化を測定して，(5)フィトクロム分子の機能に対するテトラピロール各環側鎖の役割を解析した。特にB環およびC環のプロピオン酸残基はPHYBとの結合および吸収変化に重要な役割を果たしていることや，D環の18位は17位より空間的に柔軟性に富んでいる事実の発見は，注目すべき業績である。

上述したように，本研究では発色団PCB及びその誘導体の一般的合成法の開発と，それらの機能解明に関して検討を行い，有機化学と分子生物学の学際領域の発展に大きく貢献する成果を挙げており，博士（理学）の学位を受けるに充分値するものと判定した。